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Desmos chinensis: A new candidate as natural antifungicide to control rice diseases

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ABSTRACT

Nineteen plant extracts from 16 medicinal plants were assayed for their antifungal activity against rice pathogenic fungi on PDA plates containing plant extracts. Among the plants tested, *Aegle marmelos*, *Desmos chinensis*, and *Micromelum minutum* revealed moderate to strong activities against 5 tested fungal species with more than 65.1% inhibition. The dichloromethane extract from *D. chinensis* leaves showed high antifungal activity against all 6 fungi. The dichloromethane extract possessed antifungal activity against *Bipolaris oryzae*, *Pyricularia oryzae*, and *Sclerotium rolfsii* with minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) values ranging from 3.9 to 31.2 μg/ml and 31.2 to 500 μg/ml, respectively. Bioautography assay on TLC plates with *Bipolaris setariae*, *Curvularia lunata*, *Fusarium moniliforme*, *P. oryzae*, and *S. rolfsii* displayed high antifungal activity with R_f value ranging from 0.32 to 0.37. Effects of the extract on the morphology of *B. setariae*, *C. lunata*, *F. moniliforme*, *P. oryzae*, and *S. rolfsii* were observed by scanning electron microscopy. The micrographs of mycelia and spores treated with the extract at 4MIC illustrated aberrant surface morphology and reduced conidial formation in 4 fungal species. The results demonstrated that *D. chinensis* extract possess antifungal activity against phytopathogenic fungi and the activity might lead to irreversible cellular disruption.

1. Introduction

Thailand is an agricultural country and the economically important crops are sugar cane (66 million tons), rice (31 million tons) cassava (30 million tons), and natural rubber (3 million tons) (FAO, 2010). Intensification of agriculture and monoculture of plant have increased the problems in crop cultivation including incidence of insect pests, diseases, and weeds. Diseases are among the most important limiting factors that affect rice production, causing annual yield loss conservatively estimated at 10–15% in tropical Asia (Savary et al., 2012; Mew et al., 2004).

Fungal diseases of rice include blast (*Pyricularia oryzae*), sheath blight (*Rhizoctonia solani*), brown spot (*Bipolaris oryzae*), sheath rot (*Sarocladium oryzae*), stem rot (*Sclerotium oryzae*), and black kernel (*Curvularia lunata*). Of particular importance, rice blast has re-emerged as a major factor influencing stable rice production and food security in many rice-growing countries in Asia and Africa. For example, in an average year, 50% of rice yield is lost in eastern Indian upland regions alone (Khush and Jena, 2009). The past several decades, various efforts to control plant diseases have been made at eradication or prevention through the development of synthetic fungicides (Soylu et al., 2010).

Several studies have been reported on the fungicides caused pathogen resistance, representing a potential risk for the environment, and human health (Gnanamangai and Ponmurugan, 2012). Therefore, interest in secondary metabolites from plant extracts as antimicrobial agent for use in crop protection has increased during the past decade (Wallace, 2004; Abdel-Monaim et al., 2011). Plants are rich in a wide variety of secondary metabolites which have been demonstrated *in vitro* to have antimicrobial properties. Sixteen medicinal plant species used in this study belonging to 10 families were screened against a wide range of plant pathogenic fungi including *B. oryzae*, *Bipolaris setariae*, *C. lunata*, *Curvularia oryzae*, *Fusarium moniliforme*, *P. oryzae*, *Rhizoctonia oryzae-sativa*, and *Sclerotium rolfsii*. The selection of plants for this study was based on their used in traditional medicine for the treatment of various ailments, local used as antifungal activity, ethnomedical used for microbial infections, and phytochemical consider. Medicinal plants such as *Aegle marmelos*, *Aglaia* species, *Averrhoa carambola*, *Desmos chinensis*, *Eleutherine americana*, and *Micromelum*

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Table 1
Percentage extract yield of medicinal plants used in the antifungal assay.

Botanical name	Family	Plant part tested	Extract yield (%)
Aegle marmelos (L.) Corrêa	Rutaceae	Fruit	1.70[a]
Aglaia elliptica Blume	Meliaceae	Leaf	2.50[b]
Aglaia eximia Miq.	Meliaceae	Leaf	4.24[b]
Aglaia forbesii King	Meliaceae	Leaf	6.89[b]
Aglaia forbesii King	Meliaceae	Pericarp	14.05[b]
Aglaia forbesii King	Meliaceae	Seed	3.95[b]
Aglaia grandis Korth.	Meliaceae	Leaf	5.25[b]
Aglaia odoratissma Benth.	Meliaceae	Leaf	5.18[b]
Aglaia oligophylla Miq.	Meliaceae	Leaf	10.34[b]
Aglaia pachyphyra Miq.	Meliaceae	Leaf	5.65[b]
Averrhoa carambola L.	Oxalidaceae	Root	0.77[b]
Desmos chinensis Lour.	Annonaceae	Bark	ND[c]
Desmos chinensis Lour.	Annonaceae	Leaf	7.27[c]
Eleutherine americana Merr.	Iridaceae	Bulb	4.80[b]
Horsfieldia tomentosa Warb.	Myristicaceae	Leaf	ND[b]
Micromelum minutum Wight & Arn.	Rutaceae	Root	1.84[c]
Pometia pinnata J.R. Forst. & G. Forst.	Sapindaceae	Leaf	ND[b]
Quercus infectoria Olivier	Fagaceae	Nutgall	18.5[b]
Rhodomyrtus tomentosa (Aiton) Hassk.	Myrtaceae	Leaf	7.40[b]

ND: not determined.
[a] Acetone.
[b] Ethanol.
[c] Dichloromethane.

minutum, have been reported to exhibit antifungal activity due to alkaloids (Chakthong et al., 2012), triterpenoids (Joycharat et al., 2008), anthraquinones (Jabbar et al., 1995), benzoate esters (Tuntipaleepun et al., 2012), naphthoquinones (Kusuma et al., 2010), and coumarins (Siridechakorn et al., 2012), respectively. In this study, we first screened for antifungal activity of the selected plants. *D. chinensis* was demonstrated to be the potent and good antifungal activity against all the tested fungi. Therefore, it was further observed by scanning electron microscopy.

2. Materials and methods

2.1. Test microorganisms

Rice fungal pathogens including *B. oryzae* NPRC 701, *B. setariae* NPRC 711, *C. lunata* NPRC 721, *C. oryzae* NPRC 731, *F. moniliforme* NPRC 741, *P. oryzae* NPRC 751, *R. oryzae-sativae* NPRC 761, and *S. rolfsii* NPRC 771 were isolated from naturally infected rice plants. Identifications of the purified cultures were carried out according to the cultural properties, morphological, and microscopical characteristics (Mew and Gonzales, 2002). Stock cultures of each isolate were maintained on potato dextrose agar (PDA; Difco Laboratories, USA) at 4 °C.

2.2. Preparation of medicinal plants extracts

Sixteen medicinal plants belonging to ten families were used in this study (Table 1). Parts of plants were collected on the basis of traditional practices by Thai herbalists. Botanical identification of the plant materials was obtained. A classified reference voucher specimen was deposited at the Herbarium of Prince of Songkla University, Songkhla, Thailand. Samples of selected plant species were washed with distilled water, dried in the oven at 60 °C, for 2 days, and then crushed using an electrical blender. Powdered samples were extracted in different solvents. Most were ethanol extracts. Ethanol is the most common solvent used due to its non-toxic property. However, after we tried different solvents, we found that solvents with low polarity (acetone and dichloromethane) gave compounds with high antifungal activity. The extraction period was 7 days. Three repeated extraction were performed. Powdered samples were filtered through Whatman No. 3 filter paper (Whatman Int. Ltd., UK) and evaporated under reduced pressure in a rotary evaporator (BUCHI Rotavapor R-114, Switzerland) and kept at 4 °C. The percentage yield of the extracts was obtained by dividing the final weight of the dried extract with the weight of plant material × 100. All extracts were dissolved in dimethyl sulphoxide (DMSO; Merck, Germany) before use.

2.3. Preliminary test for antifungal activity

The antifungal activities of the extracts were assessed by inhibition of mycelial growth test (Rios et al., 1988). The extracts were dissolved in DMSO and 200 μl of each extract was added to PDA to achieve final concentration at 200 μg/ml. A 5 mm agar disc containing mycelia of the test fungi was transferred to the center of the PDA plate containing the extract. Plates were incubated at 25 ± 2 °C for several days, depending on the growth of the fungi in the control plates. Propiconazole (Irvita plant protection, Netherlands) was used as the standard antifungal drug and DMSO was used as negative control. Radial growth was assessed by measuring the distance from the edge of the inoculum plug to the advancing margin of the colony. Growth inhibition of treatment against control was calculated (Eq. (A)) acording to Gamliel et al. (1989). Each experiment was conducted twice in four replicates.

$$\text{Percentage growth inhibition} = 100 - \left[\left(\frac{R^2}{r^2}\right) \times 100\right] \quad \text{(A)}$$

where R and r represent the radius of fungus colony in the treated and control plates, respectively.

2.4. Thin-layer chromatography bioautography

Thin-layer chromatography (TLC) bioautography assay was applied to detect biological activity compounds with antifungal activity present in plant extracts (Scher et al., 2004). The extracts were dissolved in hexane at a concentration of 10 mg/ml. Ten microlitres of this solution was applied on silica gel TLC plates (silica gel 60 F_{254} (0.2 mm thick); Merck). TLC plate loaded with the extract was developed in a hexane:ethyl acetate (8:1, v/v) solvent system and thoroughly dried for complete removal of the solvents. Overlay media was distributed over the developed TLC plates. After solidification, the chromatograms were inoculated with agar disc containing mycelia of the test fungi and incubated at 25 °C for 2–5 days. Clear inhibition zones were indicative of antifungal activity of the compounds separated on TLC plates.

2.5. Antifungal susceptibility testing

Broth microdilution method (CLSI, 2008) was used to determine the minimum inhibitory concentration (MIC) of the extracts which exhibited high activity against all tested fungi. Stock solution of the extracts was diluted in Roswell Park Memorial Institute (RPMI) 1640 medium with L-glutamine and without bicarbonate (Sigma–Aldrich, USA), buffered to pH 7.0 with 0.165 M 3-(*N*-morpholino)-propanesulfonic acid (Applichem, Germany) and supplemented with 2% glucose. The extract (50 μl) was diluted to final concentrations ranging from 1.9 to 1000 μg/ml in 96-well microtitre plates. The same volume of each mycelial or spore suspension containing approximately 10^4 CFU ml was inoculated and incubated at 25 °C for 24–72 h. Propiconazole was used as the standard antifungal drug and DMSO was used as negative control. MIC was observed at least in duplicate as the lowest concentration that completely inhibited visible growth. To determine a minimum

Table 2
Antifungal activity of crude medicinal plant extracts against fungal pathogens of rice.

Plant species	Mean of percentage mycelial inhibition ± standard error							
	B. oryzae	*B. setariae*	*C. lunata*	*C. oryzae*	*F. moniliforme*	*P. oryzae*	*R. oryzae-sativa*	*S. rolfsii*
Aegle marmelos	ND	25.8 ± 0.6^{g}	80.9 ± 0.2^{cd}	78.2 ± 1.4^{d}	ND	67.5 ± 1.6^{cd}	65.1 ± 0.0^{d}	92.1 ± 0.2^{b}
Aglaia eliptica	ND	63.2 ± 2.8^{e}	25.4 ± 0.5^{l}	1.6 ± 0.5^{p}	ND	40.6 ± 0.0^{fg}	10.8 ± 0.9^{i}	50.2 ± 0.8^{hi}
Aglaia eximia	ND	48.6 ± 2.4^{f}	30.1 ± 1.2^{k}	54.1 ± 0.9^{f}	ND	46.1 ± 0.8^{e}	41.6 ± 2.4^{f}	78.0 ± 0.3^{d}
Aglaia forbesii (leaf)	ND	54.0 ± 2.5^{f}	2.2 ± 1.3^{n}	11.8 ± 0.6^{q}	ND	17.2 ± 3.8^{i}	19.6 ± 1.0^{h}	0.0 ± 0.0^{m}
Aglaia forbesii (pericarp)	ND	ND	74.7 ± 0.3^{e}	44.2 ± 0.4^{k}	ND	14.6 ± 1.4^{i}	0.0 ± 0.0^{j}	50.6 ± 0.4^{hi}
Aglaia forbesii (seed)	ND	ND	86.4 ± 0.2^{b}	52.2 ± 0.4^{gh}	ND	43.7 ± 1.3^{ef}	ND	38.6 ± 0.5^{j}
Aglaia grandis	ND	74.2 ± 3.0^{c}	55.2 ± 0.4^{f}	57.7 ± 1.2^{f}	ND	43.7 ± 1.3^{ef}	7.9 ± 0.0^{i}	24.9 ± 0.8^{l}
Aglaia odoratissma	ND	12.7 ± 4.8^{h}	79.0 ± 0.5^{d}	45.8 ± 0.4^{jk}	ND	38.9 ± 1.7^{g}	34.6 ± 2.9^{g}	70.7 ± 0.6^{e}
Aglaia oligophylla	ND	ND	47.0 ± 0.5^{h}	29.2 ± 0.9^{m}	ND	75.9 ± 0.9^{a}	ND	65.3 ± 0.5^{f}
Aglaia pachyphyra	ND	66.8 ± 2.8^{cde}	52.2 ± 0.4^{g}	34.7 ± 0.4^{l}	ND	40.4 ± 0.9^{fg}	5.5 ± 0.6^{ij}	61.6 ± 0.7^{fg}
Averrhoa carambola	ND	55.5 ± 1.7^{f}	41.6 ± 1.1^{i}	ND	ND	ND	53.7 ± 1.1^{e}	29.1 ± 0.9^{k}
Desmos chinensis (bark)	ND	ND	48.6 ± 0.4^{h}	78.7 ± 0.5^{d}	ND	31.5 ± 1.1^{h}	71.1 ± 2.1^{c}	53.3 ± 0.9^{h}
Desmos chinensis (leaf)	70.6 ± 1.3^{b}	67.2 ± 4.1^{de}	97.2 ± 0.0^{a}	93.4 ± 0.7^{ab}	83.3 ± 2.6^{b}	64.4 ± 0.9^{d}	90.1 ± 2.8^{b}	99.6 ± 0.4^{a}
Eleutherine americana	ND	88.7 ± 0.2^{b}	47.0 ± 0.8^{h}	85.7 ± 0.3^{b}	ND	76.2 ± 0.7^{a}	58.5 ± 1.0^{e}	59.3 ± 5.7^{g}
Horsfieldia tomentosa	ND	54.0 ± 2.0^{f}	82.8 ± 2.6^{c}	50.6 ± 0.7^{hi}	ND	43.0 ± 0.8^{ef}	0.0 ± 0.0^{j}	59.2 ± 0.4^{g}
Micromelum minutum	ND	66.1 ± 3.1^{cde}	74.2 ± 0.5^{e}	48.2 ± 1.9^{ij}	ND	68.7 ± 0.9^{c}	75.8 ± 0.5^{c}	80.3 ± 0.0^{d}
Pometia pinnata	ND	94.7 ± 0.2^{ab}	36.0 ± 0.0^{j}	75.5 ± 1.1^{e}	ND	70.1 ± 0.6^{b}	30.3 ± 5.3^{g}	86.8 ± 0.2^{c}
Quercus infectoria	ND	3.8 ± 1.0^{i}	7.0 ± 3.1^{m}	4.9 ± 0.5^{o}	ND	0.0 ± 0.0^{j}	29.1 ± 2.7^{g}	0.0 ± 0.0^{m}
Rhodomyrtus tomentosa	ND	72.8 ± 3.0^{cd}	49.4 ± 0.6^{gh}	59.1 ± 2.0^{f}	ND	33.7 ± 1.3^{h}	56.1 ± 3.6^{e}	46.6 ± 1.2^{i}
Propiconazole	98.6 ± 0.1^{a}	98.0 ± 0.4^{a}	98.8 ± 0.0^{a}	98.8 ± 0.0^{a}	98.5 ± 0.0^{a}	98.7 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
1% DMSO	0.0 ± 0.0^{c}	0.0 ± 0.0^{i}	0.0 ± 0.0^{n}	0.0 ± 0.0^{o}	0.0 ± 0.0^{c}	0.0 ± 0.0^{j}	0.0 ± 0.0^{j}	0.0 ± 0.0^{m}

ND: not determined.
The results are means ± standard errors of four replications. Means within a column indicated by the same letter were not significantly different according to Duncan's multiple range test at the level $P < 0.05$.

fungicidal concentration (MFC), 100 μl from each of the wells at or above the MIC was plated on PDA and incubated at 25 °C for 72 h. MFC was defined as the lowest concentration at which no colonies were detected on the agar plate.

2.6. Determination of the effects of the extracts on hyphal morphology

The effects of the extracts on hyphal morphology of *B. setariae*, *C. lunata*, *F. moniliforme*, *P. oryzae*, and *S. rolfsii* were observed using a scanning electron microscopy (SEM). Fungal cells were treated with the extracts at 4MIC concentration in RPMI 1640 medium at 25 ± 2 °C for 24 h. The mycelia treated with 1% DMSO were used as control. Specimens were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3 for 2 h at room temperature. After being washed with the buffer, the specimens were post-fixed in 1% osmium tetroxide in the same buffer for 1 h at room temperature. The specimens were dehydrated in a series of graded ethanol series (50%, 60%, 70%, 80%, 90%, and 100%) for a period of 15 min in each series and dried in critical point drier. The fixed materials were mounted on stubs and coated with gold particles. Morphological changes of the fungal cells were observed by SEM Quanta 400, FEI at 10 kV.

2.7. Statistical analysis

Data on effects of the extracts on the growth of pathogens was analyzed by one-way analysis of variance and comparison of means using the Duncan's Multiple Range Test at the level $P < 0.05$. The statistical analysis was performed using statistical package for the social sciences 15.0 software for Windows (SPSS Inc., Chicago, IL, USA).

3. Results and discussion

3.1. Preliminary test for antifungal activity

A total of nineteen medicinal plant extracts, one acetone extract, three dichloromethane extracts, and fifteen ethanol extracts were screened in this study (Table 2). Out of this total, 14 extracts exhibited at least activity against two or more tested fungi with mycelial inhibition above 50%, at a concentration of 200 μg/ml. Among these, the dichloromethane extract from *D. chinensis* leaves demonstrated the broadest spectrum of activity against the tested fungi with percentage mycelial inhibition values ranging from 64.4% (*P. oryzae*) to 99.6% (*S. rolfsii*). However, only two plant extracts from *A. marmelos* and *M. minutum* revealed moderate to strong activities against 5 tested fungal species with more than 65.1% and 66.1% inhibition, respectively. Ethanolic extracts of *E. americana* and *Pometia pinnata* exerted a potential antifungal effect against *B. setariae*, *C. oryzae*, *P. oryzae*, and *S. rolfsii* with their respective radial growth inhibition percentages of 58.5–88.7% and 70.1–94.7%. Furthermore, extract of *Aglaia odoratissma* and *D. chinensis* (bark) displayed remarkable activity in the assays against 2 tested fungal species with more than 70.7% and 71.1% inhibition, respectively. Conversely, *Aglaia forbesii* (leaf) and *Quercus infectoria* extracts presented a weak inhibitory activity against all filamentous fungi assayed. Moreover, microscopic examination of white colonies revealed almost complete absence of conidia. In addition, even at concentrations that caused less than 100% mycelial growth inhibition, reduced production of pigment with loss of the characteristic dark color in *B. setariae*, *C. lunata*, and *C. oryzae*. It has been claimed that fungal melanin play a protective role against environmental stresses (Duran et al., 2002) and resistance to antifungal drugs (Da Silva et al., 2006).

3.2. Thin-layer chromatography bioautography

Bioautography was used to detect the numbers and localization of antifungal compounds in *D. chinensis* extract. Clear inhibition zones of mycelial growth and sporulation were observed against *B. setariae* ($R_f = 0.32$), *C. lunata* ($R_f = 0.32$), *F. moniliforme* ($R_f = 0.32$), *P. oryzae* ($R_f = 0.35$), and *S. rolfsii* ($R_f = 0.37$), suggesting that the substances responsible for the antifungal activity are non-polar (Fig. 1). This activity may be attributed to the presence of high concentration of flavones and benzoate esters (Rittiwong et al., 2011; Zore et al., 2011). Inhibition of benzoate para-hydroxylase (Podobnik et al., 2008), futile proton pumping that depletes ATP

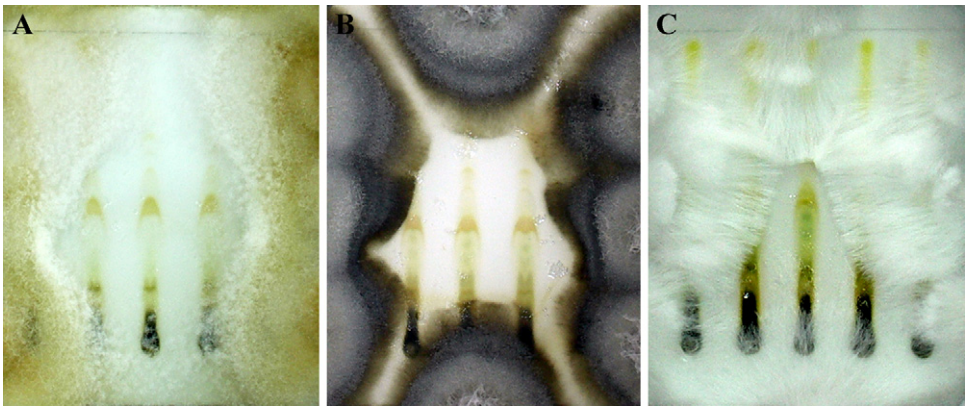

Fig. 1. Bioautography on silica TLC plates demonstrated antifungal activity of *Desmos chinensis* extract. The plates were inoculated with *Fusarium moniliforme* (A), *Pyricularia oryzae* (B), and *Sclerotium rolfsii* (C).

stores (Lambert and Stratford, 1999), and alterations in membrane permeability (Holyoak et al., 1999) by these compounds have been reported as main mode of action.

3.3. Antifungal susceptibility testing

Antifungal activities of the medicinal plant extracts expressed as MIC and MFC values against the tested fungi were assessed (data not shown). The results demonstrated that dichloromethane extract from *D. chinensis* leaves possessed good antifungal activity against all the tested fungi with MIC and MFC values ranging from 3.9 to 125 μg/ml and 31.2 to 1000 μg/ml, respectively (Table 3).

Table 3
Minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of the dichloromethane extract from *Desmos chinensis* leaves against rice fungal pathogens using broth microdilution method.

Fungal species	Dichloromethane extract MIC/MFC (μg/ml)	Propiconazole MIC/MFC (μg/ml)
Bipolaris oryzae	31.2/500	0.2/0.2
Bipolaris setariae	125/1000	0.5/0.5
Curvularia lunata	125/1000	0.5/0.5
Curvularia oryzae	62.5/500	0.5/0.5
Fusarium moniliforme	125/1000	0.5/0.5
Pyricularia oryzae	3.9/31.2	0.2/0.2
Sclerotium rolfsii	15.6/31.2	1.9/1.9

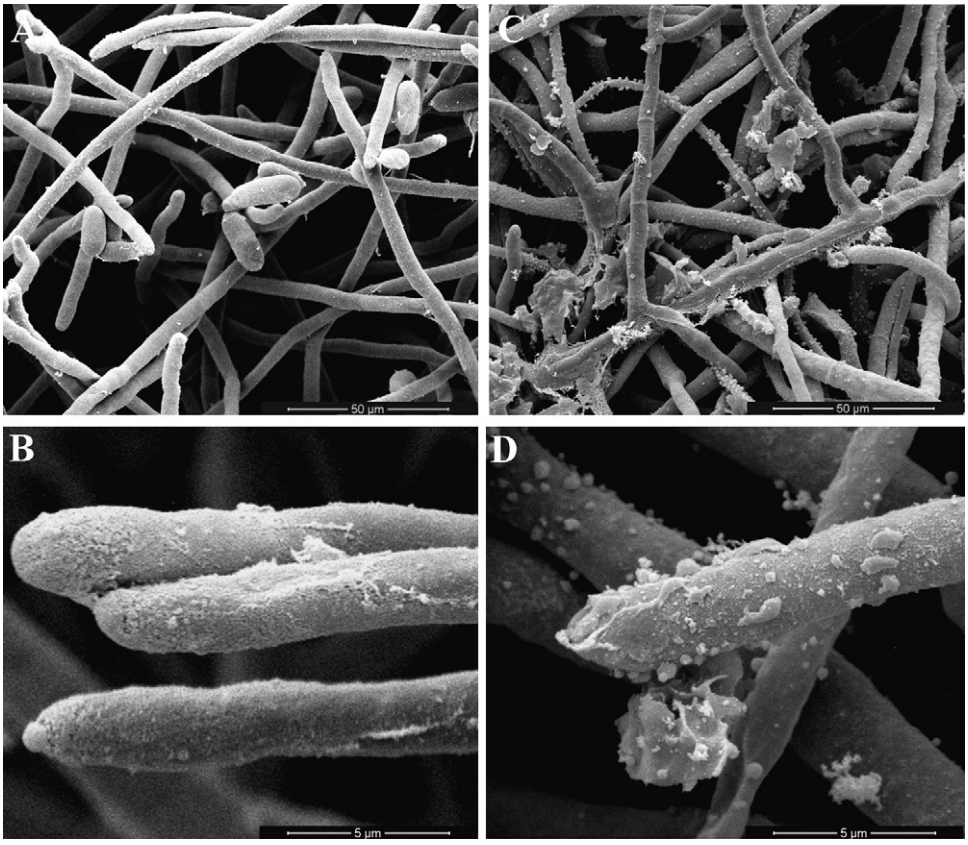

Fig. 2. Scanning electron microscope images of *Bipolaris setariae* treated with 1% DMSO (A and B) and *Desmos chinensis* extract at 500 μg/ml (C and D).

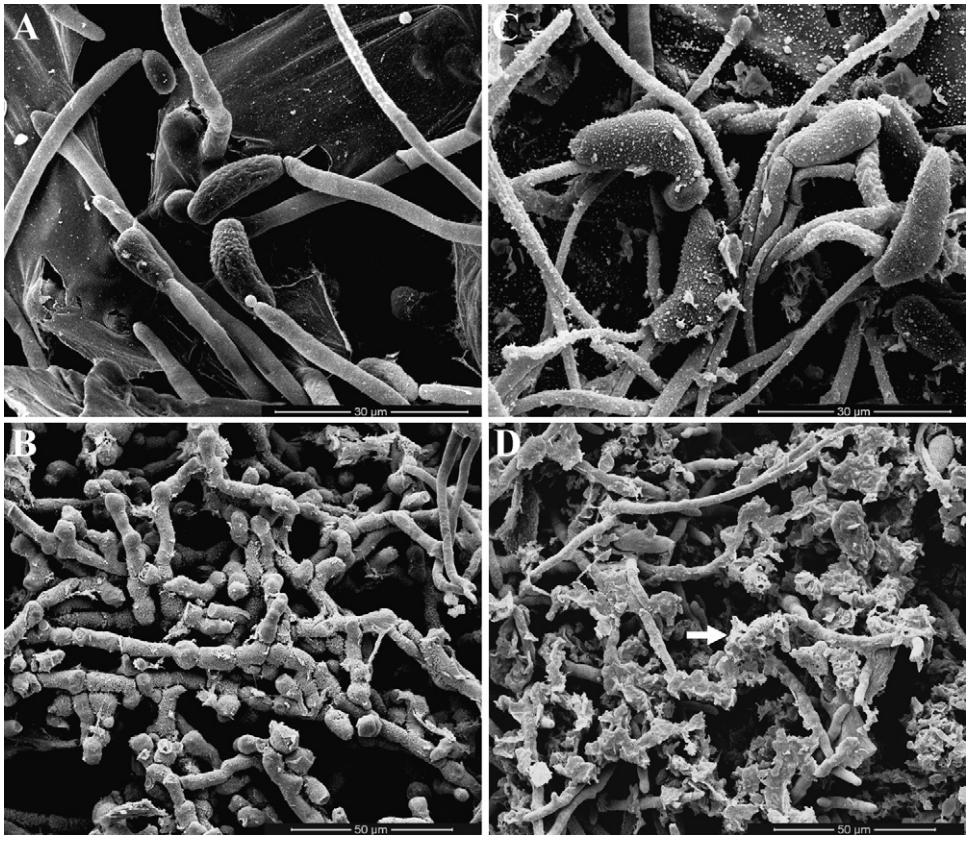

Fig. 3. Scanning electron microscope images *Curvularia lunata* treated with 1% DMSO (A and B) and *Desmos chinensis* extract at 500 μg/ml (C and D).

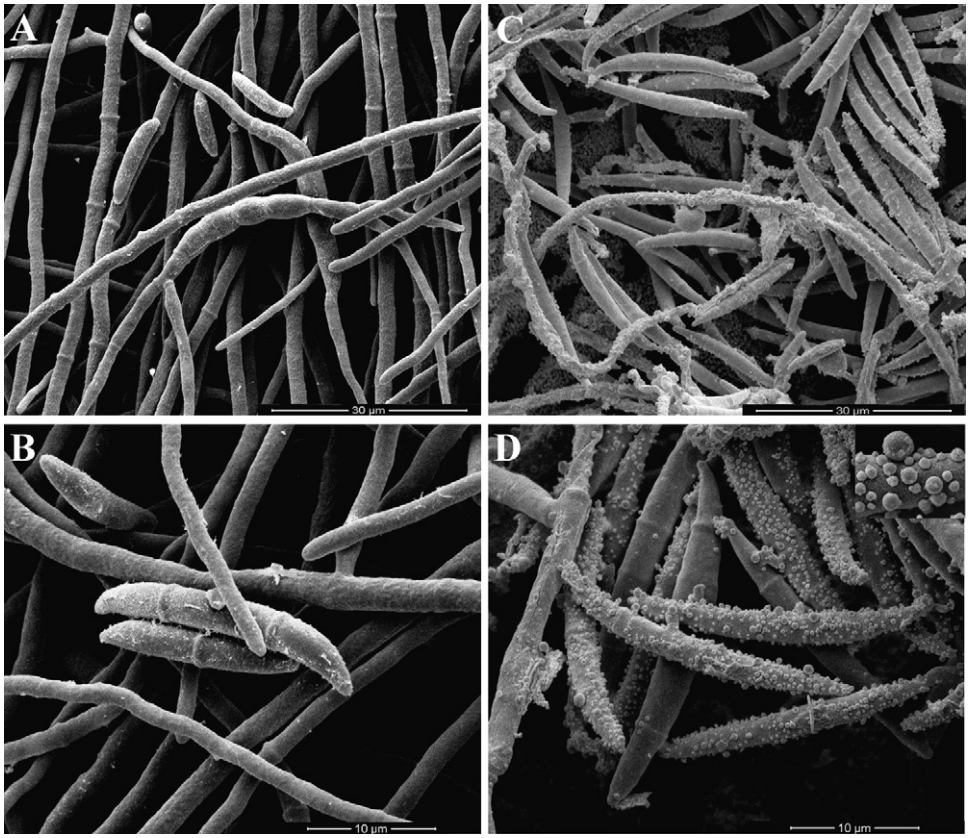

Fig. 4. Scanning electron microscope images of *Fusarium moniliforme* treated with 1% DMSO (A and B) and *Desmos chinensis* extract at 500 μg/ml (C and D).

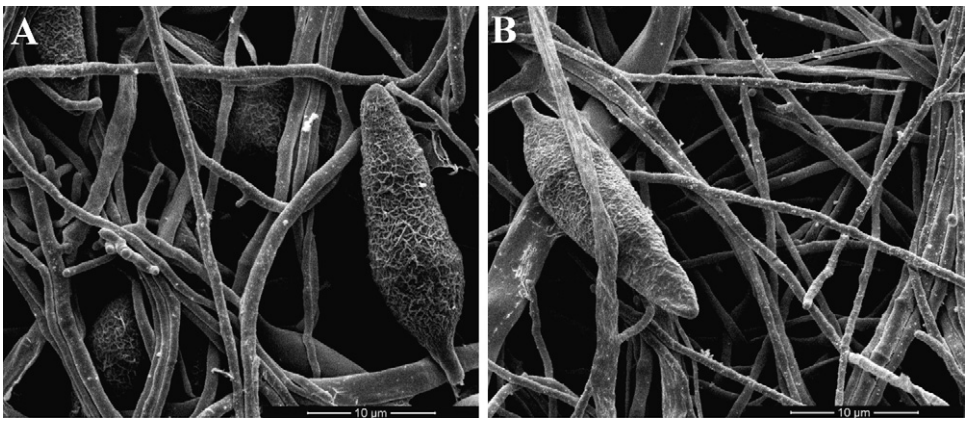

Fig. 5. Scanning electron microscope images of *Pyricularia oryzae* treated with 1% DMSO (A) and *Desmos chinensis* extract at 15.6 μg/ml (B).

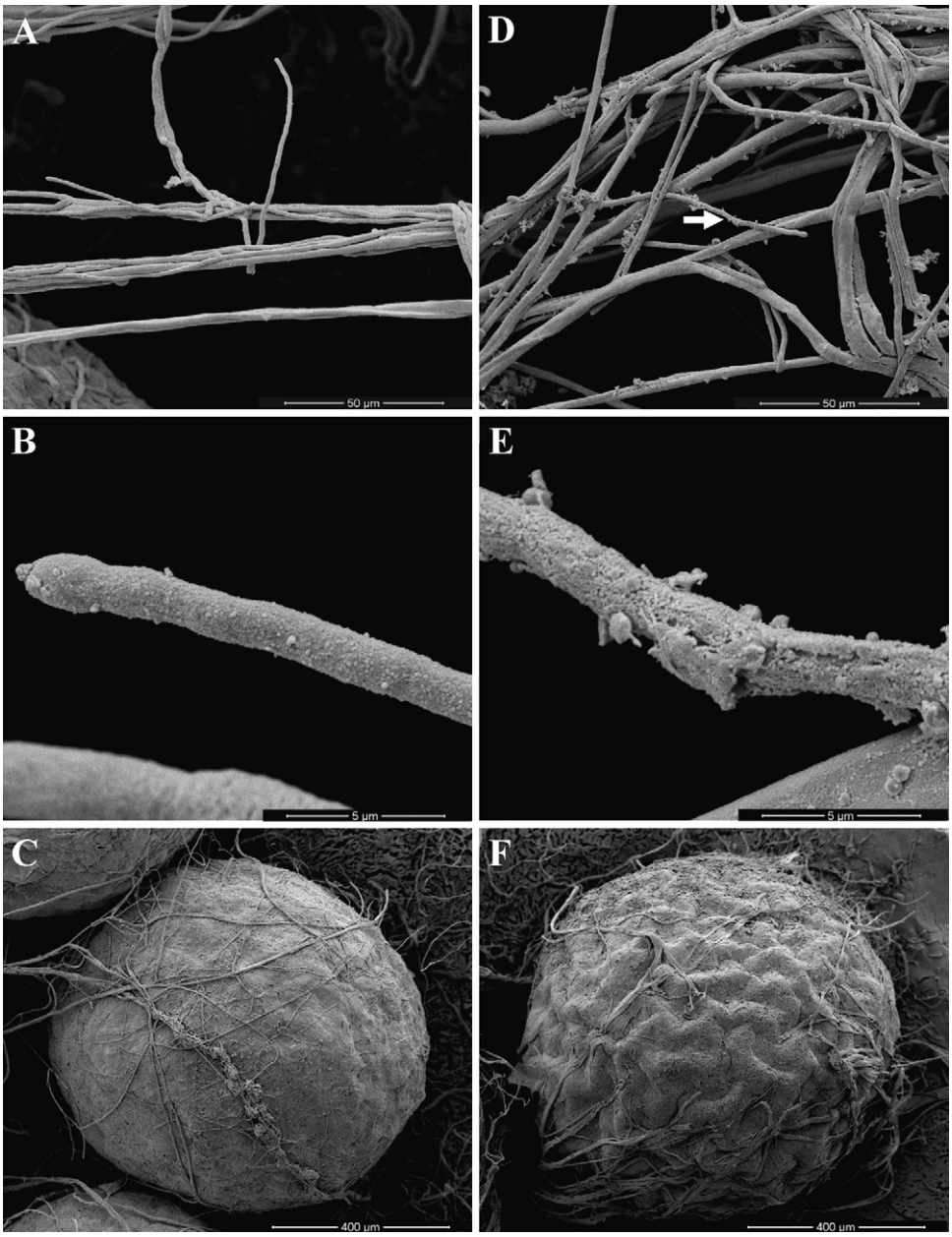

Fig. 6. Scanning electron microscope images of *Sclerotium rolfsii* treated with 1% DMSO (A–C) and *Desmos chinensis* extract at 62.5 μg/ml (D–F).

The highest level of activity was observed against *P. oryzae*, showing a MIC value of 3.9 μg/ml which was 16 times propiconazole. MIC values against *B. oryzae*, and *S. rolfsii* ranged from 15.6 to 31.2 μg/ml, whereas the MFC was found to be two-fold greater than the inhibitory concentration (except *B. oryzae*). *B. setariae*, *C. lunata*, and *F. moniliforme* were moderately susceptible with MIC value of 125 μg/ml and MFC value of 1000 μg/ml. The findings are consistent with data obtained from previous studies (Kummee and Intaraksa, 2008). Practically, a plant extract is applied in the field at concentration much higher than its MIC. It is to be noted that the results from toxicity test (data not shown) demonstrated that concentration higher than 200 mg/ml of the extract applied to rice still produced no toxicity.

3.4. Effect of D. chinensis extract on cell morphology

The micrographs clearly demonstrated that the growth of fungi in media containing the extract at 4MIC for 24 h caused profound changes in all tested fungi cell morphology. *B. setariae* mycelium grown in RPMI-1640 medium as control displayed characteristic morphology with lengthened, regular, homogenous hyphae of constant diameter with smooth external surface, and with rounded apex (Fig. 2A and B). Following the treatment with the extract at 500 μg/ml (Fig. 2C and D), the prominent morphological changes appeared rough with wrinkle cell surfaces and complete inhibition of conidia production. Similarly, the results obtained for *C. lunata* showed that the effect of extract led to the appearance of a small number of protuberances or malformed hyphae, leading to collapse and causing chlamydospores disruptions (Fig. 3C and D) in comparison to thick, elongated, and smooth surfaced in control (Fig. 3A and B). Fig. 4A and B shows images of *F. moniliforme* mycelia culture in the control, demonstrating healthy hyphae and conidia. Treatment with the extract at 500 μg/ml clearly showed distorted mycelium, rough and wrinkles on the cell surfaces, and globular structures of various sizes along the surface of the mycelia and macroconidia (Fig. 4C and D). When *P. oryzae* was exposed to the extract at 15.6 μg/ml, the hyphae showed aberrant morphology such as shrinkage and partial distortion (Fig. 5B) compared to smooth surface of untreated cells (Fig. 5A). Whereas untreated cells exhibited normal, smooth surfaces (Fig. 6A–C), the cell walls of *S. rolfsii* that had been incubated with extract at 62.5 μg/ml revealed a series of dramatic alterations including surface roughening and disruption (Fig. 6D and E) and wrinkling of the sclerotial rind (Fig. 6F). Distorted mycelium, rough and wrinkles on the cell surfaces, and reduced hyphal diameters were commonly observed in the extract treated mycelium, compared with thick, elongated, normal mycelia growth in controls. Alterations and damage on vegetative hyphae or conidia have been previously described by many workers (Phongpaichit et al., 2005; Hashem, 2011; Mares et al., 2004). However, SEM observation on rice fungal pathogen has not yet reported. The impacts of the extract on fungal structures have been suggested to be due to alterations in membrane permeability by high concentrations of benzoic acid derivatives (Wu et al., 2000), benzoate ester derivatives (Van Kiem et al., 2005), *C*-benzylated chalcones (Rahman et al., 2003), biflavones (Rittiwong et al., 2011), flavones, and oxoaporphine alkaloids (Lui et al., 2004). Electron microscopic study of fungal cells clearly demonstrated that the active constituents in the extract may interfere with fungal cell surface or endomembranes system, which affects fungal morphogenesis and growth (Amborabé et al., 2002). Ethnobotanical data documented that the use of *D. chinensis* as folk medicines by local populations for the treatment of malaria (Kakeya et al., 1993), parturition, vertigo (Rahman et al., 2003), pyretic, dysentery (Bunyapraphatsara and Chokchaichareonporn, 2000), antirheumatic, antispasmodic, and analgesic (Loi, 2001).

4. Conclusions

Preliminary data from our research on screening of the selected 16 plant species indicated that *D. chinensis* leaf extract possessed significant and broad antifungal property against a wide range of fungi. However, these results are laboratory findings which will call for field investigation to facilitate the practical use and compounds are also required further study to elucidate their antifungal mechanisms for commercial use in agricultural field.

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